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Animal Model of Human Disease

Infection-Induced Struvite Urolithiasis in Rats

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Biologic Features

Urolithiasis affects approximately 12% of the American population,[1] and struvite stones ($MgNH_4PO_46H_2O$) account for 15% to 20% of all urinary calculi.[2] Struvite calculi have also been referred to as infection stones because urease-splitting bacteria (usually Proteus, Pseudomonas, Klebsiella and Staphylococcus) are associated with struvite urolithiasis. Successful control of infection stones requires a complete understanding of the etiology, pathogenesis, and response to treatment.

High incidences of struvite urolithiasis have been reported in some inbred strains of rats;[3-5] however, there appears to be an association between presence of calculi and neoplasia of the bladder and ureter. Miniature schnauzer dogs were reported to have a high incidence of spontaneous urolithiasis,[6] and calculi can be experimentally produced in miniature schnauzers and beagles.[7] Bladder stones were produced in rats by implanting bacterial contaminated foreign bodies (zinc discs and silk sutures) within the bladder; however, systemic infection and severe kidney damage was observed.[8]

We employed a modified procedure described by Satoh et al[9] to produce infectious struvite urolithiasis in outbred male or female Sprague-Dawley rats (CRL:CD(S-D)BR) with a greater than 95% success rate.[10] Two zinc discs (0.36 ± 0.05 cm) were implanted in the bladder of halothane anesthetized rats through a small bladder incision, and the bladder incision was closed with one 4/0 chronic gut suture. The suprapubic incision was closed with 4/0 chronic gut sutures and autoclips. Seven days later, anesthetized rats were urethrally catheterized with lubricated polyethylene tubing (PE 10), and 0.2 ml containing 10^7 Proteus mirabilis organisms were deposited in the bladder.

Progressive calculus formation was observed on the zinc disc with an accumulation of 0.019 ± .004 g/disc of stone material 24 hours after inoculation increasing to 0.075 ± .057 g/disc in 7 days (Figure 1). Rats examined 30 days after inoculation had large bladder calculi (0.13 ± .09 g/disc), bilateral hydronephrosis, pylonephritis, thickened bladder walls, and cystitis. Bladder stones, staghorn calculi in the renal pelvis (Figure 2), and occasionally ureter calculi were observed in rats killed 30 days after infection. The stones were determined to be composed of struvite with traces of apatite ($Ca_{10}(PO_4)_6CO_3$) by chemical analysis and electron probe X-ray analysis. Examination of the stones by transmission electron microscopy revealed bacterial microcolonies sandwiched between crystalline areas (Figure 3).

Comparison with Human Disease

The stones recovered from this model are biochemically and microscopically indistinguishable from those recovered from patients with infectious struvite urolithiasis.[11,12] In this model the rats developed azotemia, alkaline urine, and hematuria. Urease positive bacteria (Proteus mirabilis) were found in the urine and within the calculi as is observed in patients with struvite urolithiasis. Humans and rats in this model both develop struvite uroliths in the bladder, renal pelvis, and ureter; pylonephritis, hydronephrosis, and cystitis are associated with the infectious stone. Patients with struvite urolithiasis require removal of the calculi as bacteria within the stone matrix are notoriously resistant to antibiotic therapy. Morphologic examination of struvite stones by scanning and transmission electron microscopy of struvite stones recovered from patients or experimentally infected rats showed that struvite calculi contain large numbers of

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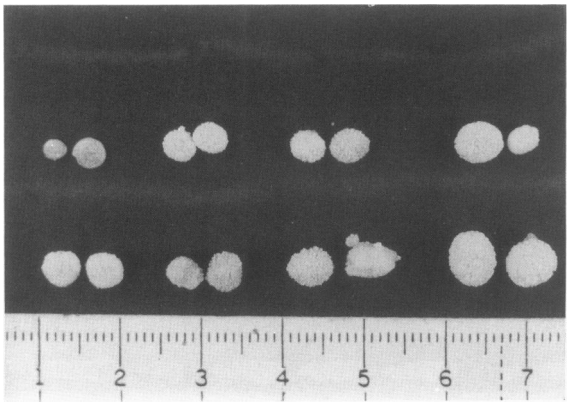

Figure 1. *The two zinc discs removed from the bladder of rats killed on day 0 through 7 after inoculation of bladder with Proteus mirabilis. This demonstrates the progressive calculus formation on the zinc disc surfaces (scale is in cm).*

bacterial cells and their products. The failure of antibiotic therapy has been investigated and is believed to be primarily due to the extensive glycocalyx production by adherent microcolonies of bacteria.[13,14]

Potential Usefulness of the Model

We used this model to study the role of bacteria in the formation of struvite uroliths in the bladder and renal pelvis. This model can also be used for testing antimicrobial agents, urinary acidifiers, and urease inhibitors as therapeutic agents for infectious urolithiasis. This rat model may also be employed as a model for calculi associated hydronephrosis, pylonephritis, ureteritis, and cystitis.

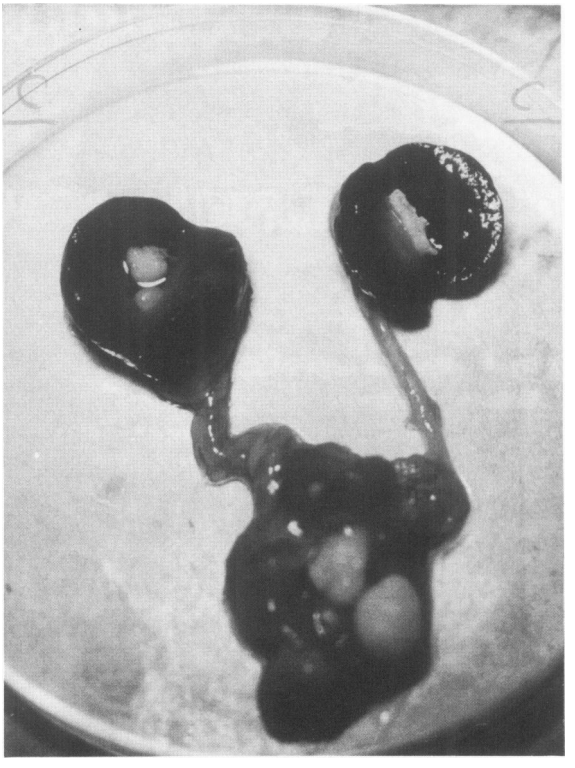

Figure 2. *Rats killed 30 days after bacterial inoculation had developed bilateral renal calculi, bladder stones, and bilateral hydronephrosis.*

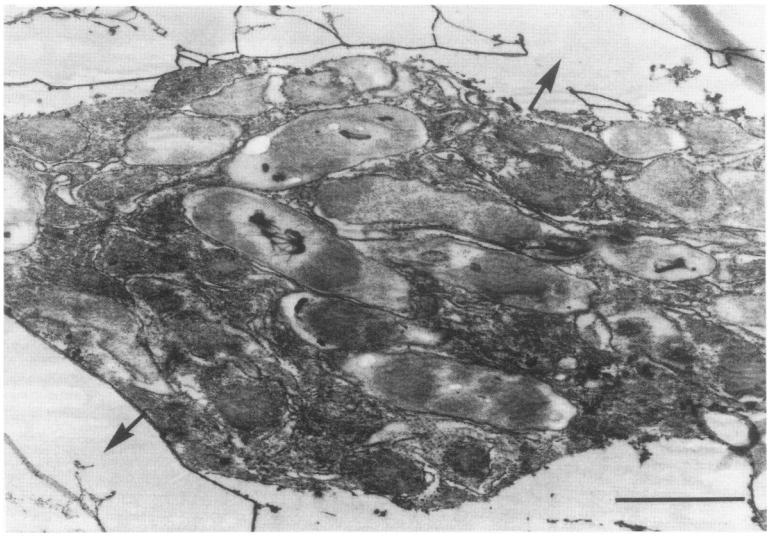

Figure 3. *TEM of material scraped from the surface of a zinc disc 168 hours after bacterial inoculation. A microcolony of gram-negative rods is sandwiched between crystalline areas (arrows). Bar, 1 μ.*

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